HISTOPHYSIOLOGICAL STUDIES ON THE CORPUS ALLATUM OF LEUCOPHAEA MADERAE. III. THE EFFECT OF CASTRATION [1, 2]

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In the insect, *Leucophaea maderae,* and in related species reproductive processes are controlled by an intricate neuroendocrine mechanism (Scharrer, 1958a, 1959; Engelmann, 1960). Each reproductive cycle is initiated by a hormone from the corpora allata. Concurrently with alternating phases of ovarian activity and quiescence, the corpora allata become active and inactive at regular intervals. They remain inactive as long as they are restrained by nervous stimuli from the brain. The reactivation of the corpora allata at the appropriate time is controlled by a variety of factors, among them feed-back from the reproductive organs. The exact nature of this feed-back is still unknown, but it seems that a humoral factor is involved that acts by way of the central nervous system. As a rule, corpus allatum activation occurs when the ovaries contain undeveloped eggs, except during "pregnancy," *i.e.*, as long as an ootheca is present in the uterus. The active corpus allatum, found in animals with growing eggs, shows a considerable increase in volume and other distinct changes in its histology as compared with the inactive gland characteristic of newly emerged or pregnant animals (Engelmann, 1957; Scharrer and von Harnack, 1958).

The individual components of this neuroendocrine control system can be analyzed by experimental interference at various levels, for example, by severance of the nerves restraining the corpora allata (Scharrer, 1952), or by gonadectomy (von Harnack and Scharrer, 1956). Such procedures disrupt the normal functional and structural periodicity of the corpora allata.

In the absence of the ovaries, the corpora allata of *Leucophaea* continue in a state of activity beyond the normal period. The question arises whether this condition surpasses the normal degree of activity of the corpus allatum cells, and whether the lack of intermittent inactivation of the gland may perhaps result in permanent structural change or even damage.

The present paper deals for the most part with a morphological analysis of the corpus allatum of gonadectomized females of *Leucophaea* ranging in age from emergence to senescence. The situation in males seems less interesting, since their corpus allatum fails to respond to gonadectomy as in females.

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Material and Methods

The present study includes 154 adult females and 26 males of the roach, *Leucophaea maderae*, gonadectomized under varying environmental conditions in our laboratories in Cleveland, Denver, and New York between 1944 and 1959. The large majority of the females (147) were operated upon at various nymphal stages, the remaining seven shortly after they had become adults. In the ovariectomized nymphs, the interval between operation and emergence ranged from 1 to 152 days. In the group operated in the adult stage, the longest interval between emergence and castration was 18 days. The completeness of the gonadectomy was verified by examination of the excised tissues under the dissecting microscope, by testing the experimental animals individually for possible reproduction by placing them in separate jars containing normal males, and by autopsy at the time of fixation. The condition of the accessory sex glands was also recorded; excessive amounts of secretory material are characteristic of gonadectomized females.

Fixation was scheduled at intervals ranging from an adult age of nine to 457 days. The material thus encompasses the entire adult life span. The total period after gonadectomy, therefore, ranged from a minimum of 32 to a maximum of 491 days. The majority of the animals (143) had been castrated for over 100 days.

A smaller number of males was included in this study; their corpora allata are more uniform in structure, and presumably less complex in function, than those of females. All of the 26 male castrates were operated upon as nymphs, 12 to 104 days before emergence. The adult age at the time of fixation varied between 0 and 366 days. The total castration period thus ranged from 39 to 432 days, with all except three specimens falling in the group above 100 days.

The histological procedures and the methods of quantitative evaluation of the corpora allata were essentially the same as those described in the first paper of this series (Scharrer and von Harnack, 1958). Most cases were fixed in Helly's solution. The Halmi-Dawson "aldehyde fuchsin" method was used with the addition of Weigert's hematoxylin as a nuclear stain. The present study includes tissues treated with the standard periodic acid-Schiff (PAS) technique. The sections were kept in the periodic acid solution and in the Schiff reagent for 10 minutes each. The duration of pretreatment with buffered saliva ranged from 30 minutes to two hours. Certain additional specimens were fixed in 10% formalin, alcohol-formol-acetic acid (AFA), or Carnoy's solution.

Among the female castrates, 57 representative cases were selected for complete quantitative analysis, *i.e.*, volumes of the corpora allata, nuclear numbers, and nuclear-cytoplasmic ratios were determined. In the remaining 98 animals only volumetric determinations were made. Since organ volume, accompanied by histological inspection of the relative amount of cytoplasm, is a good indicator of the degree of activity of the corpora allata, these specimens serve to substantiate the conclusions drawn from the study of the more completely analyzed group.

In the males, volumetric determinations were used as an indication of corpus allatum activity, since the normally low variability of their corpora allata was not significantly altered after gonadectomy. In the largest and smallest glands of this group, nuclear numbers and nuclear-cytoplasmic ratios were calculated in order to delimit the range of variability within the male castrates.

Additional methodological matters, such as hemicastration, will be mentioned in the text.

Results

a. Females

When the volumes of the corpora allata of all gonadectomized females studied were plotted according to adult age (Fig. 1), several facts became apparent.

All age groups with the exception of the youngest and the oldest showed a considerable spread in corpus allatum size. Starting from glands whose volume

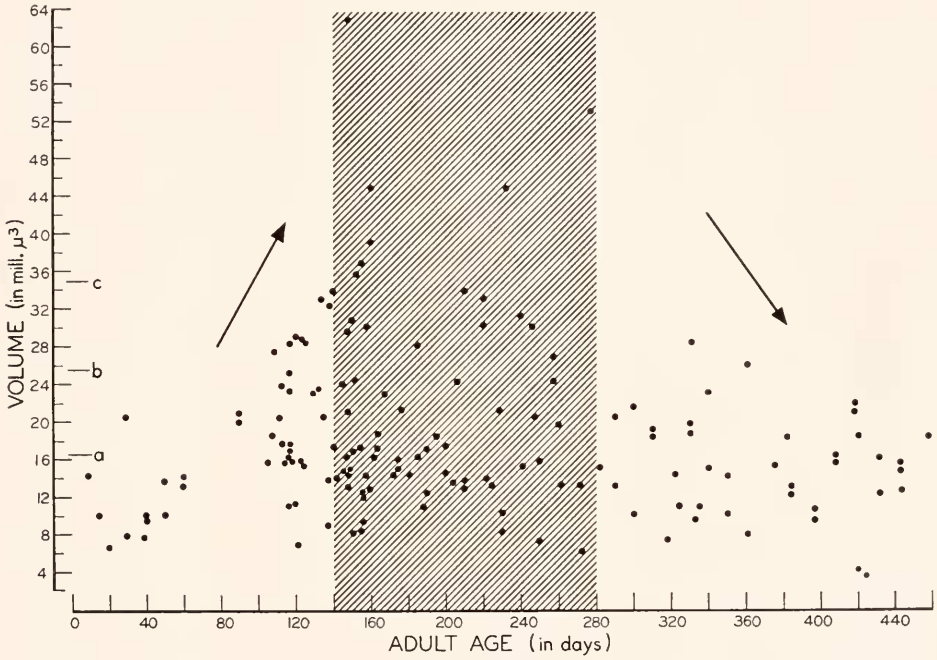


Figure 1. Diagram showing the size variation of corpora allata of 154 ovariectomized *Leucophaea* arranged according to adult age. Each dot represents the measurement of the paired glands from one individual. The arrows indicate that, with increasing age, the volumetric maxima rise stepwise to reach peak values in a mid-period (140–280 days; shaded area), and gradually decline in old age. For comparison, three maximum levels of corpus allatum size in groups of non-castrate animals are indicated on the scale: a, First activity cycle preceding first ovulation of normal controls; b, Second activity cycle following first parturition in normal controls; c, Response to feeding following drastic starvation.

matches that of the smallest inactive glands in normal controls, through specimens appearing moderately or fully active, the range extends to organs much larger than any observed in the previous studies of this series.

These supernormal values seem to be unique for gonadectomized females. They are, therefore, of primary interest, even though their number in the present series is comparatively small. A total of 26 animals surpasses the maximum size of the corpora allata characteristic of the second reproductive cycle of normal controls.

Of these, seven exceed also the higher level reached by females which had been returned to a normal diet after prolonged starvation (von Harnack, 1958). The possible reasons for the scarcity of excessively high corpus allatum values as well as the great variability of corpus allatum size within age groups will be discussed below.

If one follows, in consecutive age groups, the distribution of the maxima in corpus allatum size a pattern becomes apparent which is indicated by the arrows in Figure 1. Comparable to the situation in normal post-emergence females, there is, with increasing adult age, a gradual volumetric increase. However, in contrast to the intact animals with their predictable periodic return to corpus allatum inactivity, the growth trend continues for a longer time in the ovariectomized animals. At about 100 days of adult age, their highest value for the corpus allatum reaches the limit typical of the second reproductive cycle of normal females. One hundred and forty days after emergence the maxima in the castrates match the still higher top level of those females which had received a normal diet after drastic starvation (von Harnack, 1958).

The "giant" corpora allata, found so far exclusively in castrate females, all occur in the age group between 140 and 280 days. After this period the maxima gradually decline throughout the remaining adult life span whose termination is represented by a female killed at the high adult age of 455 days.

The interpretation of these volumetric data should be based on the following considerations. Our previous studies of the activity cycles of the corpora allata of *Leucophaea* have shown a remarkable capacity of these glands to change their structure according to physiological conditions, a responsiveness which expresses itself by alternating volumetric increase and decrease. It is difficult to assess the range of individual variation, both as to the degree of response and the time required by each specimen to react in a given situation. Since this variability from individual to individual exists in normal animals kept under uniform laboratory conditions (Scharrer and von Harnack, 1958), one may expect it to be even more pronounced in experimental animals such as the starvation series (von Harnack, 1958) or in the present, in which the normal cyclic pattern of the corpora allata has been disrupted by the removal of an important target organ, the ovary.

Furthermore, the value obtained for a given specimen fixed at any given age does not permit conclusions as to its past history or (theoretical) future potentiality. For example, small corpora allata in an animal of—let us say—200 days of adult age may have become hyperactive early, as did those of the female fixed 148 days after emergence, and may subsequently have regressed. It is equally possible that a sample represents a slowly responding type which would have eventually reached a peak of the kind indicated by the excessively large gland fixed at the adult age of 280 days. At the time of fixation, a corpus allatum devoid of feedback stimuli from the ovary may even have passed through more than one phase of activation. This possibility is suggested by the observation that corpus allatum implants free from central nervous control may change from inactivity to activity and vice versa (Engelmann, 1960). Certain as yet undetermined extrinsic or intrinsic conditions may modify the effect of castration on the corpus allatum (see also Doane, 1960). Finally a specimen, for reasons unknown, may have been altogether unresponsive to the abnormal situation created by gonadectomy.

In view of all these considerations, it is not surprising that a relatively large number of cases in the present experimental series show corpora allata that lie within the normal range of volumetric variation. Theoretically, the chances of "hitting" a gland at its peak value are small, especially if the state of maximal activation were of short duration.

Thus it is the occurrence rather than the frequency of abnormally high volumetric values which is of primary significance. Their existence demonstrates the capacity of the corpus allatum of *Leucophaea* to respond to abnormal physiological states which, in degree, far surpasses even that observed under drastically changed nutritional conditions. The most plausible explanation for this pronounced histophysiological response in gonadectomized specimens seems to be the persistent lack of ovarian feed-back stimuli. The prolonged effort of the corpora allata to stimulate a target organ which is no longer present presumably results in morphological changes indicative of hyperfunction.

The assumption made here, that corpus allatum volumes are not maintained at peak levels indefinitely, is supported, but not proved, by the absence of abnormally high volumetric values among all gonadectomized females of the series whose adult life span surpasses 280 days. Since no periodic changes in afferent stimuli, like those from gonad and ootheca in normal females, can account for the inactivation of the corpora allata of castrates, one may conclude that states of excessive glandular stimulation eventually subside on their own account.

The next question of interest concerns the changes in nuclear-cytoplasmic ratio in relation to corpus allatum volume (Fig. 2). It may be recalled that, as a general rule established in normal adult females, the number of nuclei per unit of corpus allatum tissue decreases with increasing organ volume. One may inquire whether the same holds for the corpora allata of gonadectomized females and, if so, whether the shift in nuclear-cytoplasmic ratio continues in those female castrates whose corpus allatum volume surpasses the maxima of the normal physiological range.

In all those gonadectomized females whose corpus allatum size, at the time of fixation, was within that of the normal physiological range, the relationship between organ volume and nuclear-cytoplasmic ratio compared well with that in unoperated controls. In the smallest corpus allatum from a castrate animal (3.6 million μ^3) the number of nuclei per mm.^{2} was calculated as 17,251. In a control gland of similar size (3.4 million μ^3) the corresponding figure was 16,765. In both the experimental and the normal series, these figures gradually decreased with increasing organ size to about one fourth of the initial level. In the castrates, glands with volumes paralleling the upper limit of the normal physiological range had, for example, 4389 nuclei per mm.^{2}, the corresponding count for normal specimens being 3770 nuclei per mm.^{2}. Young and old gonadectomized females with corpora allata of similar size did not show differences in nuclear-cytoplasmic ratios. Thus, generally speaking, the level of "activity," as determined by the histological criteria discussed so far, is approximately the same in pairs of specimens with comparable corpus allatum size, irrespective of age, or the presence or absence of ovaries.

If the trend in shifting nuclear-cytoplasmic ratios were to continue at the same rate beyond the confines of the physiological size range, the relative cytoplasmic content and thus the degree of "activity" in the largest corpora allata of the gonadectomized series would indeed be unusual.

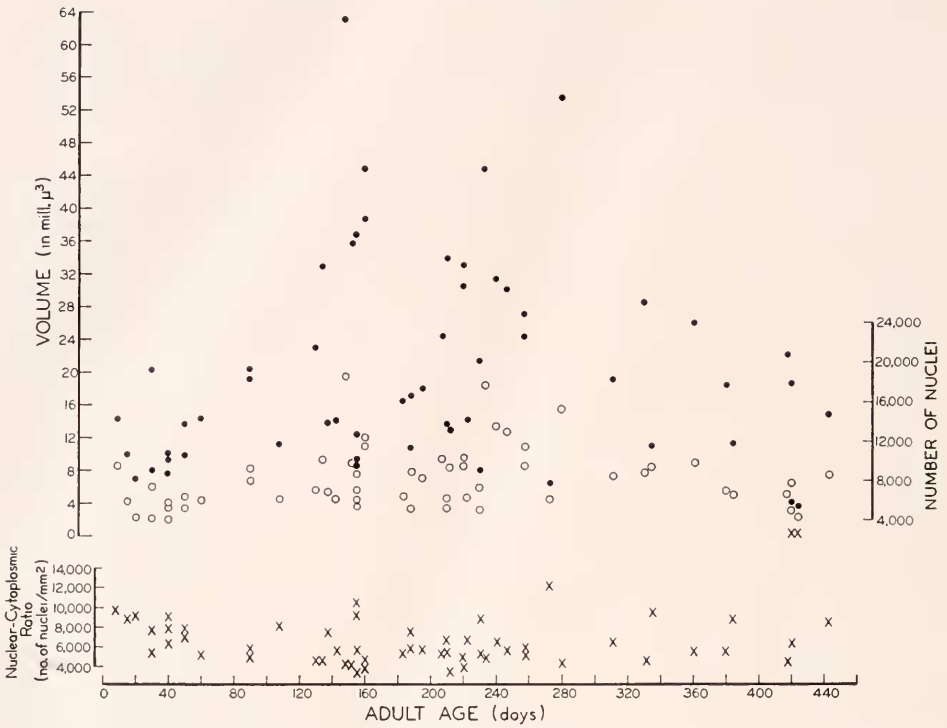


FIGURE 2. Diagram summarizing quantitative analysis of corpora allata of 57 samples of ovariectomized *Leucophaea* selected so as to illustrate the range of variation throughout the adult life span. ● = volume of both corpora allata; o = number of nuclei calculated for both glands; x = nuclear-cytoplasmic ratio.

The following data show that this is not the case. The smallest figure, 3486 nuclei per mm.², found among the oversized corpora allata of the castrates, is not significantly lower than the minimum of the control series (3698 nuclei per mm.²). Therefore, one must conclude that the increase in relative cytoplasmic content with rising corpus allatum size reaches a plateau at a volumetric level equal to the maximum of the control group. Beyond this point, there seems to be no more drastic change in nuclear-cytoplasmic ratio, a situation which is also demonstrated by the fact that the nuclear counts in gonadectomized specimens continue to rise

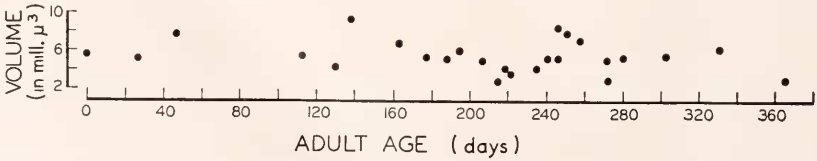


FIGURE 3. Diagram showing the volumetric values of corpora allata of 27 gonadectomized males. Compared with the situation in females, no clear-cut castration effect is noted. The range of variation is small and bears no relationship to adult age.

with increasing corpus allatum volume. They reach a maximum (18,736), which considerably surpasses that in normal controls.

Thus it appears that the increase in corpus allatum volume following ovariectomy, once it reaches abnormal proportions, differs from the growth process signalling activation of the gland in the course of its normal functional cycles. In the normal gland, the increase in nuclear number is surpassed by that in cytoplasmic content. By contrast, both nuclear number and cytoplasmic content increase proportionally during the period of overgrowth of the corpora allata of castrates.

In accordance with their considerable size variation, the corpora allata of castrate females differ widely in their morphology. As may be expected, they lack clear-cut signs of cyclic activity such as those that can be identified in normal females (Scharrer and von Harnack, 1958). Thus, for example, mitotic figures and pycnotic nuclei, although not frequent, occur throughout the life span with the exception of very young castrate adults.

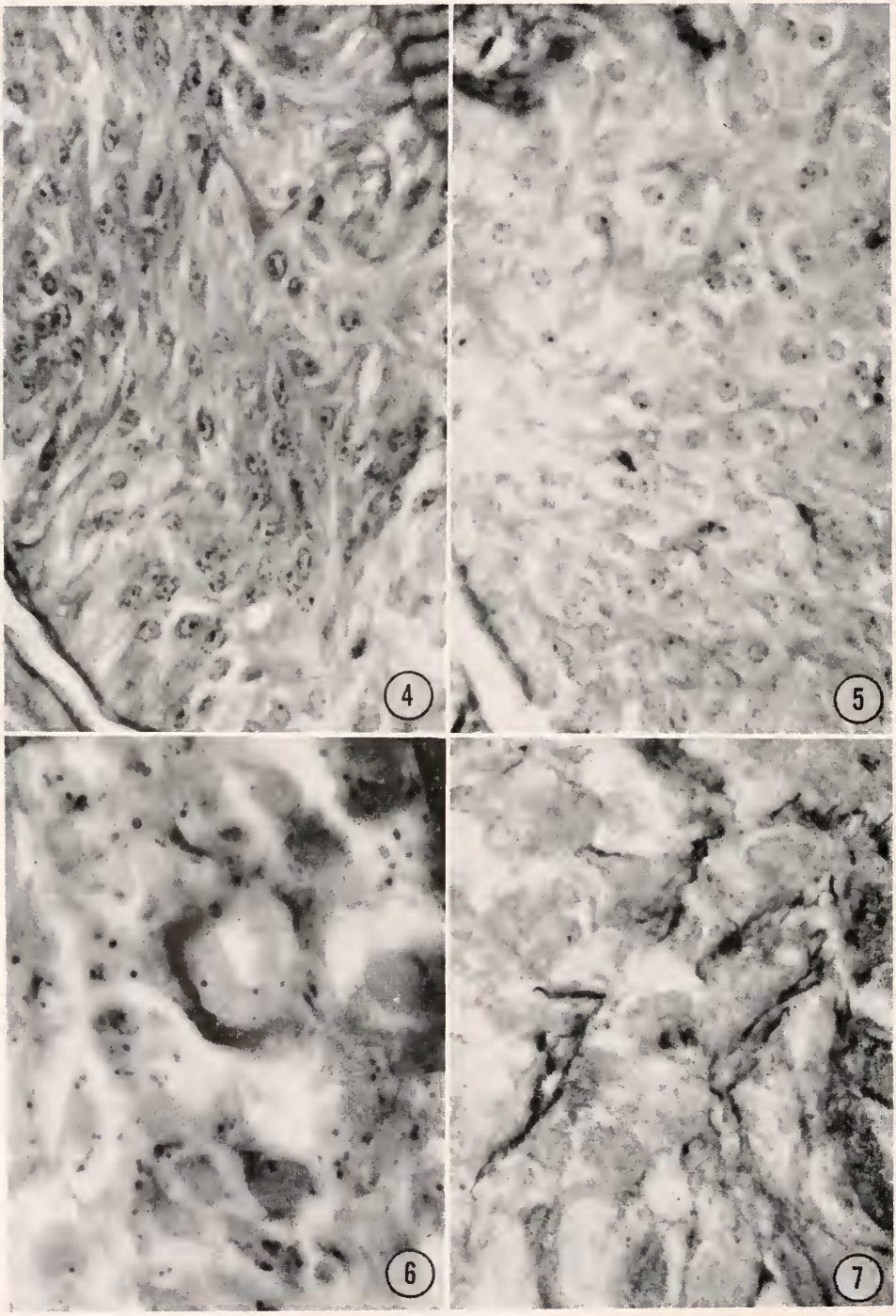
Aside from this apparent difference in periodicity, the quantitative data as well as histological features presented so far indicate that, within a comparable volumetric range, the glands of castrates resemble those of normal controls in several ways. A small corpus allatum, be it from a newly emerged normal or an old gonadectomized animal, has densely packed nuclei and shows very little cytoplasmic detail. With increasing size of the corpora allata conspicuous histological changes which are related to the gradually shifting nuclear-cytoplasmic ratio occur in both the normal and the experimental groups.

Cells filled with small, aldehyde fuchsin-positive granules, similar to those described in normal animals (Scharrer and von Harnack, 1958), are found in many of the castrates, especially those with medium-sized and large corpora allata. As a rule such cells are not numerous, and a careful search of serial sections is necessary for their detection. The youngest castrate female in which such cells were recorded had been killed 30 days after emergence. From this point on no preferential age distribution of positive cases was apparent. The present study did not add anything to the understanding of the functional role of these cells. The possibility that they are hemocytes is being explored.

More important in the corpus allatum cells of castrate as well as normal females are inclusions which stain lavender or pale green with the Halmi-Dawson method used. They are present after fixation in Helly's as well as formalin, AFA, and Carnoy's solution. In saliva-treated PAS preparations these granules stain intensely positive. This and the results with the other techniques used so far suggest that they belong to the category of glyco- or mucoproteins.

Matched pairs of experimental and normal animals show the following relationships. In younger castrates the PAS-positive granules are not conspicuous; they compare in number and size with those of normal controls. However, at an adult age of two months and over, the amount of this material in castrates with good-sized corpora allata significantly surpasses that in unoperated females (Figs. 6 and 7).

The examination of several other tissues revealed that this phenomenon is not restricted to the corpus allatum (Scharrer and von Harnack, 1960). In the fat body, the difference between ovariectomized and normal specimens is considerable. The wide range of possible variation in the content of PAS-positive, saliva-resistant material is illustrated by Figures 10 and 11. It should be stated, however,



FIGURES 4–7.

that not all castrates reach the degree of accumulation shown in Figure 10, while on the other hand normal controls may contain more inclusions than those depicted in Figure 11. In spite of these variations, there can be no doubt that the average amount of this material is considerably increased several months after gonadectomy.

Comparable PAS-positive inclusions are also noticeable in the muscles of gonadectomized females with an adult age of over 40 days. They are particularly conspicuous in areas near the insertion of a muscle fiber in the chitinous skeleton (Fig. 12). In normal animals very few or no such inclusions are observed in the musculature.

Another example is the central nervous system where castrates also show a greater amount than normal females of PAS-positive material other than glycogen. This material is seen in varying amounts within numerous ganglion cells. However, the most pronounced difference occurs in a special type of neurosecretory cell (B cell) of the subesophageal ganglion which normally contains only few granules in the periphery of the perikaryon (Fig. 9). With few exceptions, in castrates this pair of cells accumulates large amounts of cytoplasmic granules which stain intensely with the PAS technique (Fig. 8). In Halmi-Dawson preparations these granules are distinguished by their green coloration. These cells have been called "castration cells" in an earlier study (Scharrer, 1955). Within the limited scope of the histological tests employed so far, the inclusions in the fat body, musculature, and nervous tissue resemble those of the corpora allata. Morphologically they differ in that they are larger.

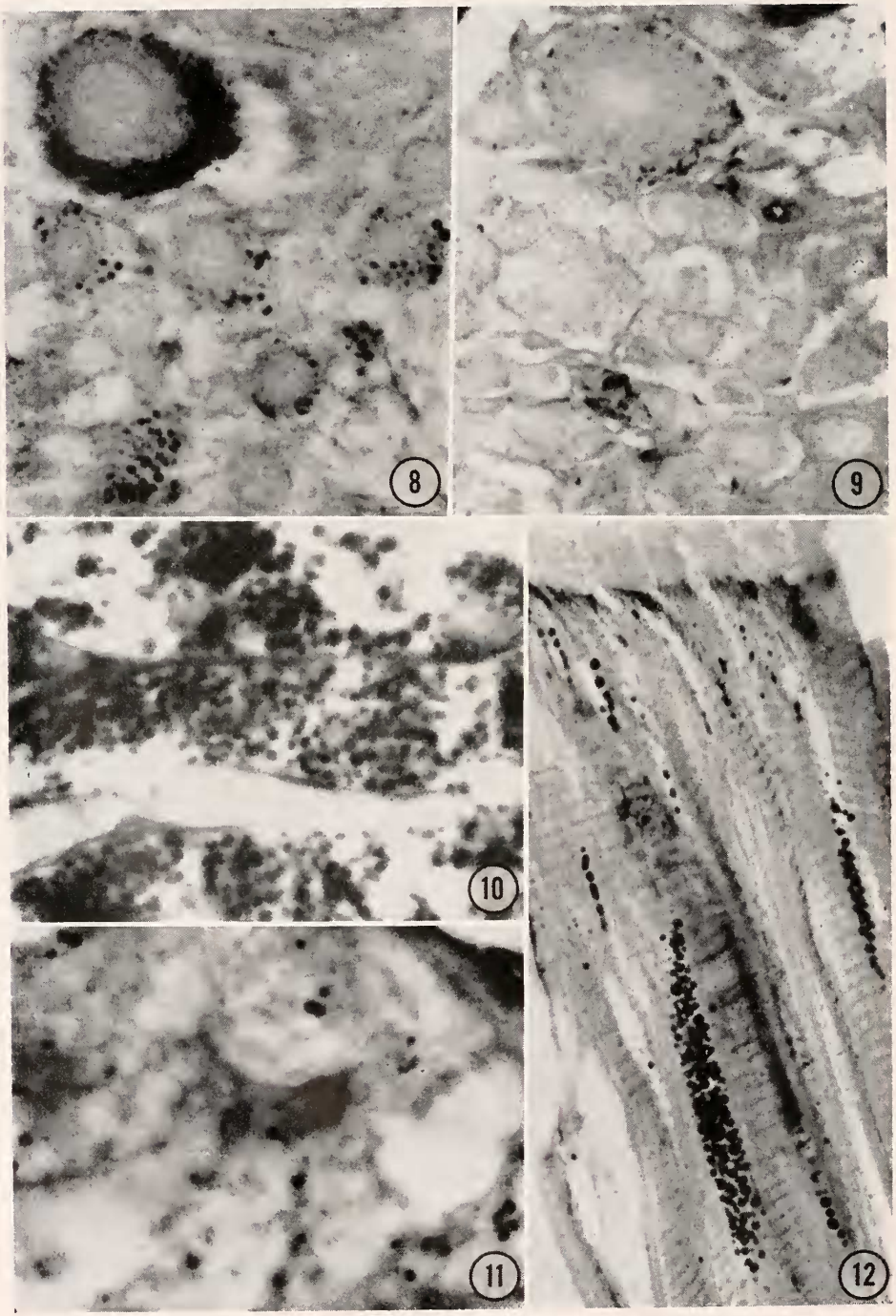
Sections of corpora allata with high cytoplasmic content may show varying degrees of vacuolization. Also, individual cells may appear partially separated from each other and thus more easily identified than in smaller specimens where cell boundaries can be detected only in electron micrographs. When the glands reach abnormally high volumes in castrates, the form and arrangement of individual cells undergo further characteristic changes. The orientation of cell processes toward the periphery of the gland and the probable relationship of this arrangement to the release of secretory material into the surrounding hemolymph in highly active normal corpora allata have already been reported (Scharrer and von Harnack, 1958). In the largest castrate specimens, many cells appear "stretched" to the point of becoming spindle-shaped. The resulting pattern of corpus allatum structure is quite characteristic (compare Figs. 4 and 5). To what extent this might

FIGURE 4. Section through corpus allatum of gonadectomized female of *Leucophaea*. Adult age 148 days; volume of corpora allata 62.9 million μ^3 (maximal value of series). Note characteristic pattern of spindle-shaped cells. Helly, 7 μ, modified aldehyde fuchsin. $\times$ 450.

FIGURE 5. Control section for Figure 4; same technique. Active corpus allatum of normal female fixed at adult age of 125 days; 15 days after parturition; ovarian eggs nearly mature; volume of corpora allata 18.7 million μ^3. Note irregular arrangement of cells (the existence of cell boundaries, difficult to see in the light microscope, will be demonstrated in a future publication on the ultrastructure of the corpus allatum). $\times$ 450.

FIGURE 6. Section through corpus allatum of castrate female showing numerous PAS-positive granules. In addition to these intracellular inclusions, other PAS-positive elements seen are a small part of the corpus allatum sheath (upper right corner) and intercellular material within the gland. Adult age 228 days. Formalin, 7 μ, saliva pretreatment, PAS. $\times$ 1200.

FIGURE 7. Control section, treated with same technique as in Figure 6, through active corpus allatum of normal female of same adult age; 10 days after parturition; development of ovarian ova well under way. Note relative paucity of PAS-positive granules. Intercellular elements are comparable to those in Figure 6. $\times$ 1200.



FIGURES 8–12.

be a sign of "pathological" change remains undetermined. Aside from this feature, glands of extraordinary size show few cytological peculiarities not also observed in smaller glands.

In our material several unoperated cases with a history of ovarian dysfunction, related to senility or of unknown origin, were of particular interest for comparison with castrates. As might be expected, these "normal" animals displayed several of the cytological features, though not always all, characteristic of the experimental group. For example, one specimen with the exceptional adult age of 599 days had abnormally large corpora allata (vol. 37 million μ^3, as compared with 25.5 for the maximum control value) with a normal "active" appearance except for the presence of a giant nucleus. In this case, PAS-positive granules were more abundant than normally, in the corpora allata as well as the central nervous system and the fat body, but not in the musculature. In another case, which had reached the adult age of 300 days without signs of reproductive activity, the number of granules was increased in the musculature as well as the corpus allatum and the fat body.

In view of the castration effects just described it was of interest to study the situation after unilateral gonadectomy. Fifty-two females whose right ovary had been removed before emergence produced offspring at normal intervals but, on the average, only one-half of the number of young borne by unoperated control animals (Scharrer, 1958b). In their histology, the corpora allata of this experimental group resembled those of normal adult females. According to the stage in the ovarian cycle at which the animals were fixed, the structural criteria used in this study indicated varying degrees of glandular activity. The fat body, musculature, and nervous tissue also showed no abnormalities. Thus, hemicastration apparently fails to lead to those morphological changes characteristic of females without both ovaries or with severe disturbances in gonadal function.

b. Males

The corpora allata of the 26 castrated males studied showed no drastic effects of the operation. The volumetric values in this group (range: 2.8 to 9.4 million μ^3; mean: 5.5 million μ^3) are only somewhat higher than those of 46 normal control specimens (range: 2.1 to 9.0 million μ^3; mean: 4.9 million μ^3). The total number of nuclei calculated for the smallest (3486) and the largest glands (3867) of the

FIGURE 8. Section through subesophageal ganglion of castrate female showing abundance of PAS-positive granules. These occur in various ganglion cells, but are particularly concentrated in the large B-type neurosecretory cells designated as "castration cells"; one of these in upper left corner of Figure. Same animal and same technique as in Figures 6 and 10. × 600.

FIGURE 9. Control section through same area as in Figure 8 of subesophageal ganglion of normal adult female. Note that B-type neurosecretory cell and adjacent ganglion cells contain only a few PAS-positive granules. Formalin, 7 μ, saliva pretreatment, PAS. × 600.

FIGURE 10. Section through cephalic fat body of castrate female. Same animal and same technique as in Figures 6, 8, 12. Note numerous PAS-positive granules. × 1400.

FIGURE 11. Control section for Figure 10 through cephalic fat body of normal female. Same animal and same technique as in Figure 7. In contrast to that shown in Figure 10, this specimen contains few granules. × 1400.

FIGURE 12. Longitudinal section through cephalic musculature of castrate female. Same animal and same technique as in Figures 6, 8, 10. Note conspicuous accumulations of PAS-positive granules. Normal controls show only rare granules. × 560.

gonadectomized series differs only little, and consequently the range of nuclear-cytoplasmic ratios is considerable (15,571 nuclei per mm.^{2} in the smallest, and 5117 in the largest gland). Thus the corpora allata of castrate males resemble those of normal males and females in that the relative amount of cytoplasm is increased when the glandular volume rises.

In normal adult males, fluctuations in corpus allatum volume occur during the entire adult life span (Scharrer and von Harnack, 1958). Similarly, castrates show no significant variations of "activity" as determined by organ volume and nuclear-cytoplasmic ratio during adult life (Fig. 3). Neither is there, within the range of the time intervals of the experiment, a relationship between these values and the total (nymphal plus adult) length of the castration period. The animal with the largest glands happens to have an adult age of 138 days, that with the smallest one of 273 days. It may also be of interest to illustrate the relationships between male and female castrates by selecting a comparable pair of specimens. In the male with the largest corpora allata (9.4 million μ^3) the total nuclear count was 3896 (5117 nuclei/mm.^{2}), in a female with corpora allata of the same size it was 3994 (6110 nuclei/mm.^{2}).

Inasmuch as the corpora allata of male *Leucophaea* show hardly any volumetric response to changing physiological conditions, either during the normal life cycle or following castration, no dramatic changes in glandular histology were expected. The glands from castrate males compare in their cellular structure with those of normal males and of females whose corpora allata are inactive or only mildly active. The amount of saliva-resistant PAS-positive material present in castrate males does not surpass that in normal males or females. This applies to the cell inclusions in the corpora allata as well as those in the fat body, musculature, and nervous tissue.

Discussion

The present study has demonstrated significant changes in the histology of the corpora allata in response to gonadectomy in females of *Leucophaea*. By contrast males showed at best only a very mild reaction. The latter result is in line with previous negative results in males of several other insect species (Day, 1943; Bodenstein, 1947; Johansson, 1958). The slight rise in the average volume of the corpora allata of male castrates of *Leucophaea* might be dismissed as insignificant, except that it seems to parallel a similar responsiveness in normal adults. In these, a mild stimulation of the glands occurs during the post-emergence period, *i.e.*, at a time when in normal females a much more pronounced (pre-ovulatory) activation of the corpora allata was observed (Scharrer and von Harnack, 1958). Possibly these data indicate a limited capacity in male insects to respond to changes in the internal milieu comparable to that displayed, for example, by the male mammary gland. The lack of clear-cut histological correlations between the corpora allata and gonadal activity in males of *Leucophaea* is not surprising because all other available data speak for the absence, in the male sex, of a physiological role of these glands in the control of reproduction (Scharrer, 1946). But even in a case, such as in the gregarious phase of *Schistocerca,* where "sexual maturation" in the male is controlled by the corpora allata, these glands seem unaffected by gonadectomy, at least as far as physiological criteria are concerned (Loher, 1960).

The data in the literature are less consistent regarding the situation in female insects. Several investigators noted a volumetric increase in the corpora allata

of castrates (Pfeiffer, 1940; Thomsen, 1942; Day, 1943; Bodenstein, 1947; Johansson, 1958; for indirect evidence see also Vogt, 1942; Lukoschus, 1956; Doane, 1960; Gill, 1960). Studies in other species failed to reveal increased volumes following ovariectomy (Day, 1943; Kaiser, 1949). In view of the small proportion of cases with excessively large corpora allata in the series described in the present paper and their restriction to a certain age group, it seems reasonable to suggest that in the investigations quoted above castration effects may have escaped detection. This was certainly the case in two previous papers dealing with *Leucophaea* (Scharrer, 1946; Lüscher and Engelmann, 1955).

On the other hand, species differences have to be taken into consideration. This refers not only to volumetric responses, but to the manner in which these come into effect. For example, in two Diptera, *Calliphora* (Thomsen, 1942) and *Lucilia* (Day, 1943), the higher volume in the corpora allata of castrates is due to an increase in cell size, but not in cell number, whereas in *Leucophaea* an increase in cell number, as well as cell size, in conjunction with glandular activation has been definitely established.

The possible reasons for the low frequency of corpora allata showing pronounced castration effects in our present material have been discussed earlier in this paper. The same interpretation may apply to other species for which a considerable overlap between the volumetric values of gonadectomized and normal specimens has been reported (Thomsen, 1942; Bodenstein, 1947). Similarly, according to Doane (1960) [4], the hypertrophy of the corpus allatum in the sterile mutant adp/adp of *Drosophila* is an unstable condition. These observations, in addition to the present more detailed study, make it seem unlikely that the corpora allata of ovariectomized females stay active indefinitely as was postulated for *Leucophaea* by Lüscher and Engelmann (1955).

The role of the PAS-positive, saliva-resistant granules observed in various tissues of *Leucophaea* is still uncertain. The discussion of their possible significance must take into account the following points:

(1) Cytoplasmic inclusions whose histochemical characteristics suggest that they belong to the category of glycoproteins are not restricted to the corpora allata, but occur in a variety of other tissues. Among these, the fat body, the musculature, and the nervous system have been tentatively explored in this study. The tests applied so far are too limited to permit any conclusions regarding the extent of chemical relationship among these cell inclusions. The granules occur in both sexes. Their numbers show some individual variation, presumably in relation to different physiological states. It seems possible that some of them, *e.g.*, in the corpus allatum or in the neurosecretory cells, are glycoprotein hormones or their precursors comparable to those of the delta cells of the vertebrate adenohypophysis.

(2) Attention should be focused on the fact that these "glycoprotein" granules increase in number and size in the corpora allata of gonadectomized females, as compared with normal controls. Concomitantly, the fat body, musculature, and

[4] We wish to thank Dr. W. W. Doane, Yale University, for permitting us to read her thesis before its publication. Part of it has appeared in print since the present paper went to press. See: Doane, W. W. 1960. Developmental physiology of the mutant *female sterile (2) adipose* of *Drosophila melanogaster*. I. Adult morphology, longevity, egg production, and egg lethality. J. Exp. Zool., **145**, 1–21; and Doane, W. W. 1960. Developmental physiology of the mutant *female sterile (2) adipose* of *Drosophila melanogaster*. II. Effects of altered environment and residual genome on its expression. J. Exp. Zool., **145**, 23–41.

central nervous tissue of female castrates show conspicuous deposits of this material, whereas it may be almost absent in normal animals. Furthermore, the tissues examined show a certain uniformity as to the degree of their response. A given animal with a particularly high content of inclusions in the fat body also has proportionately more of them in the other tissues involved than does another animal in which these changes are altogether less conspicuous. Another site showing castration effects of this kind is the hemolymph, but their analysis must await future study.

By contrast, male castrates do not differ from intact males with respect to the amounts of this material present in the various tissues mentioned. This difference between the sexes represents an additional illustration of an already known situation, *i.e.*, the apparent absence of a corpus allatum-gonad axis in male *Leucophaea*. Thus, the data in castrate and normal males can hardly contribute to the understanding of the functional relationships among the "glycoprotein" granules in different tissues. Our interest, therefore, centers on the more dynamic situation in the females.

Here, the marked increase of these inclusions after gonadectomy, as well as the parallelism in the response of several different types of tissue, suggests a common denominator. Obviously, the appearance of these castration effects is related to the absence of the ovaries.

One possible explanation of the phenomenon is that the increase in the number and size of these cytoplasmic inclusions in animals without ovaries is an expression of a metabolic change (see, for example, Sägesser, 1960). Perhaps "precursor materials" (Pfeiffer, 1945), normally intended for yolk production, become deposited in abnormal quantities in tissues of castrate females. If this were true in the case of *Leucophaca*, allatectomized females, in which the ovaries and accessory sex glands are arrested (Scharrer, 1946), should show an increase in cell inclusions in the fat body, musculature, etc. comparable to that in castrates. Preliminary exploration of such animals, as well as female castrates subjected to prolonged starvation, suggests that the cell deposits in question are not, or at least not exclusively, surplus metabolites and that a role of the ovary more specific than that of "depository" for yolk material may be involved. Perhaps the castration effects reported are related to the absence of an ovarian hormone, an interpretation which is also strengthened by recent observations in *Drosophila* (Doane, 1960).

The effects of gonadectomy on the corpora allata in female insects discussed here show certain parallelisms among crustaceans and vertebrates; in these both sexes are involved. Parasitic or surgical castration in isopods, in which no organ analogous to the corpus allatum is known, leads to hypertrophy of the sinus glands (Yamamoto, 1955, Oguro, 1960). In mammals, such as the rat, the absence of the gonads elicits changes in the anterior pituitary indicative of glandular hyperfunction. Among such castration effects have been reported not only hypertrophy and increased gonadotropin content, but also characteristic changes in the morphology and cytochemistry of the gonadotrophs resulting in "castration cells" (see Hellbaum and Greep, 1940; Ladman and Barrnett, 1956; Takewaki, 1956). It is of particular interest that these cells, like the corpus allatum cells of ovariectomized *Leucophaea*, contain more glycoprotein than those of normal controls (Catchpole, 1949/50; Purves and Griesbach, 1955).

Summary

1. The corpora allata of *Leucophaea maderae* display an impressive capacity to respond to alteration of the internal milieu resulting from ovariectomy. Compared with the periodic structural changes of these glands in the course of normal reproductive cycles, the morphological characteristics of the corpora allata of castrate females are quite unpredictable. There is considerable spread from low organ volumes indicating glandular inactivity to excessively high values so far unmatched in non-castrate animals.

2. Only a small proportion of ovariectomized animals have abnormally large corpora allata, and these are confined to an intermediate period of the adult life span. This suggests that their growth response lacks permanency. Its apparent cause is the persistent absence of feed-back stimuli from the ovaries. The reasons why this castration effect on the corpus allatum eventually subsides are unknown.

3. The rise in relative and absolute cytoplasmic content with increasing organ size, observed earlier in normal animals, applies equally to gonadectomized females whose corpora allata at the time of fixation did not exceed the maximum of the control values. Above this level, the growth pattern differs in that no further drastic change in nuclear-cytoplasmic ratio occurs, *i.e.*, nuclear numbers and cytoplasmic content increase at the same rate.

4. Compared to those of normal controls, the corpora allata of castrate females contain progressively increasing amounts of saliva-resistant PAS-positive granules, probably glycoprotein in nature. A conspicuous accumulation of comparable cytoplasmic inclusions also occurs in various other tissues, such as the fat body, musculature, and nervous system. The functional relationships of these variously located inclusions are still uncertain, but the parallelism in their frequency suggests a common denominator. Whether they accumulate because of the absence of an ovarian hormone, or merely in response to metabolic changes following castration, is as yet undetermined.

5. In contrast to females, male castrates of *Leucophaea* do not differ significantly from normal controls as to the volume and cytoplasmic content of their corpora allata. The same applies to the presence of PAS-positive material in various tissues. This difference between the sexes in response to castration is undoubtedly related to the absence of a corpus allatum-gonad axis in male *Leucophaea*.

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